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A COMPARISON OF THE INHIBITORY EFFECT OF CASTELLANI'S PAINT AND OF GENTIAN VIOLET SOLUTION ON THE IN VITRO GROWTH OF CANDIDA ALBICANS*

KATHLEEN RILEY, M.D. AND ARTHUR H. FLOWER, JR., M.D.

Many organic dye solutions have been used in the clinical treatment of intertriginous lesions produced or complicated by the presence of *Candida albicans*. The yeast-like organism, gives a positive staining reaction by Gram's method as evidence of its tenacious affinity for the dye gentian violet. Churchman's (1) important work showing the inhibitory effect of gentian violet on the growth of various gram positive bacteria and moulds made tenable the assumption that *Candida* and other yeasts would be therapeutically vulnerable to this organic dye. Gentian violet solutions have been used, therefore, in the treatment of common monilial infections such as thrush, erosio interdigitale, blastomycetica monilial onychomycosis, and paronychia with good clinical results in many instances.

Later, laboratory investigations provided additional support for the known clinical efficacy of gentian violet. Farley (2) studied the ability of gentian violet to act as a restrainer in the isolation of pathogenic moulds. Schamberg and Kolmer (3) investigated the fungistatic and fungicidal effect of various dyes including gentian violet. Weidman (4) included in his studies the specific effect of gentian violet and crystal violet on certain pathogenic yeasts and fungi. Gomez-Vega (5) examined the mycostatic effect of gentian violet on certain *Candida* while Sanderson and Smith (6) investigated the effect of gentian violet on the yeast-like organism causing systemic blastomycosis infection. The results obtained by these workers are in general agreement in that "in vitro" very weak dilutions of gentian violet have definite fungistatic ability.

Castellani's (7) paint, a compound solution of carbolfuchsin, is another organic dye solution which has been used widely in dermatological therapy. At the time of its introduction, Castellani stated that it was clinically effective in patients with pruritus ani and pruritus vulvae which he suspected of having "superadded saccharomycetic, cryptococcic, monilial, and bacterial infections". Very little has been written about the use of this agent in intertriginous dermatoses of monilial origin until a recent article by Seale and Clark (8). In their hands the compound solutions of carbolfuchsin proved to be effective in the treatment of intertriginous moniliasis. *In vitro* studies showed that the full strength solution was inhibitory to the growth of *Candida* but that graded dilutions of the compound solution of carbolfuchsin apparently did not inhibit as well. The exact methods used in their *in vitro* studies, however, was not presented. The com-

* From the Division of Dermatology and Syphilology of the Dept. of Medicine, Duke University School of Medicine, Durham, N. C. Dr. J. Lamar Callaway, Chief.

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ponents of the compound solution were studied individually as to their fungistatic effect and found to have only slight fungistatic quality. They asserted further that gentian violet, while not only cosmetically unacceptable, was therapeutically less effective and produced a fair number of untoward reactions. Since this was contrary to our clinical experience and unsupported by the bulk of previous *in vitro* studies, we have undertaken further careful *in vitro* studies comparing the inhibitory effect of gentian violet and compound solution of carbolfuchsin and its individual components on a known virulent strain of *Candida albicans*.

METHOD

A saline suspension of 1–1000 dilution (by volume) of *Candida albicans* was used to inoculate Sabouraud's agar culture plates. The chemical solutions and melted agar were mixed before the plates were poured. One loopful of the 1–1000 suspension of *Candida albicans* was streaked on each plate. Three plates were made up with each dilution to be tested and inoculated with the suspension for comparison purposes and to circumvent contamination failures.

The 1 per cent gentian violet in 10 per cent alcohol solution was used in dilutions of 1–10, 1–100, 1–1000, 1–10,000. The plates containing Castellani's compound solution of carbolfuchsin represented dilutions of 1–50, 1–100, 1–200, 1–300, 1–400, and 1–500.

Later, the component substances in Castellani's compound solution of carbolfuchsin were tested separately using their approximate concentrations as follows: 5 per cent aqueous solution of phenol, 8 per cent resorcin solution, 4 per cent aqueous acetone solution, 0.8 per cent aqueous boric acid solution, and 7 per cent alcoholic solution of basic fuchsin. These solutions were diluted to 1–25, 1–50, 1–100, 1–200 and tested against a loopful 1–1,000 saline suspension of *Candida albicans* on Sabouraud's agar plates. The factors used before were duplicated as nearly as possible. All experiments were accompanied by control plates, all plates were incubated at 37°C for 48 hours, and the amount of growth was noted at 24 and 48 hour intervals.

RESULT

After 24 hours incubation all plates containing the dilutions of gentian violet solution showed a complete inhibition of growth. The plates containing 1–50, and 1–100, dilution of Castellani's compound solution of carbolfuchsin also showed complete inhibition. A moderate growth of the *Candida albicans* was noted on the plates containing 1–200 through 1–500 dilution of Castellani's compound solution of carbolfuchsin. After 48 hours incubation the plates containing the gentian violet solution showed inhibition up to the 1–10,000 dilution. The plates with the Castellani's compound solution of carbolfuchsin revealed no growth in the 1–50 dilution plate but moderate growth in the 1–100 dilution plate and abundant growth in all the other plates. Tables 1 and 2, fig. 1.

Duplication of the tests using the individual constituents and slightly stronger concentrations produced the following information. Using Castellani's compound solution of carbolfuchsin as a control, identical results were obtained at both the

24 and 48 hour readings. After 24 hours incubation, the plates containing 1–25 dilution of 5 per cent aqueous solution of phenol revealed complete inhibition. The plate containing the 1–25 dilution maintained the inhibition at 48 hours. The growth on the plates with the higher dilutions was quantitatively greater at 48 hours. The plates containing the 8 per cent aqueous resorcin solution showed only slight growth in the 1–25 dilution both at the end of the 24 and 48 hour readings. The plates containing the 7 per cent alcoholic solution of basic fuchsin showed complete inhibition of growth in the 1–50 dilution plates after both the 24 and 48 hour readings. There was a moderate increase in the quantitive growth

TABLE 1

Sabouraud's agar with 1 per cent gentian violet in 10 per cent alcohol

DILUTION	24 HOURS	48 HOURS
1:10 (1:1,000 of gentian violet powder)	No growth	No growth
1:100 (1:10,000 of gentian violet powder)	No growth	No growth
1:1000 (1:100,000 of gentian violet powder)	No growth	No growth
1:10,000 (1:1,000,000 of gentian violet powder)	No growth	Slight growth
Control Plate	Abundant growth	Abundant growth

TABLE 2

Sabouraud's agar with Castellani's paint

DILUTION	24 HOURS	48 HOURS
1:50	No growth	No growth
1:100	No growth	Mod. growth
1:200	Sli. growth	Good growth
1:300	Mod. growth	Good growth
1:400	Mod. growth	Good growth
1:500	Mod. growth	Good growth
Control	Abundant growth	Abundant growth

in the 1–100 dilution at the 48 hour reading as against the 24 hour reading. The 4 per cent aqueous acetone solution and the 0.8 per cent aqueous boric acid solution failed to influence the growth in the 1–25 dilution even at the 24 hour reading. Table 3, fig. 2.

All control plates showed abundant growth of the organism at each reading.

COMMENT

For the specific species of *Candida albicans* grown on Sabouraud's agar at 37°C. our studies reveal that gentian violet has a far superior fungistatic ability when compared with Castellani's compound solution of carbolfuchsin or any of its individual ingredients. The gentian violet was effective in inhibiting growth of this species of *Candida albicans* in a 1–1,000,000 dilution. Castellani's com-

pound solution of carbolfuchsin was effective only in a dilution of 1–100. The ingredient most active in Castellani's compound solution of carbolfuchsin was the alcoholic solution of basic fuchsin beginning at a dilution of 1–100. The next most active ingredient was the 5 per cent aqueous solution of phenol which demonstrated inhibition starting at the 1–50 dilution. The other components of the solution exhibited very poor or no inhibition at the concentrations studied.

From these studies there is very little to explain the reported efficacy of using Castellani's compound solution of carbolfuchsin in the treatment of intertriginous moniliasis. Although the inhibitory effects of the solution are poor, it must be recognized that clinical effectiveness may be explained by some of its

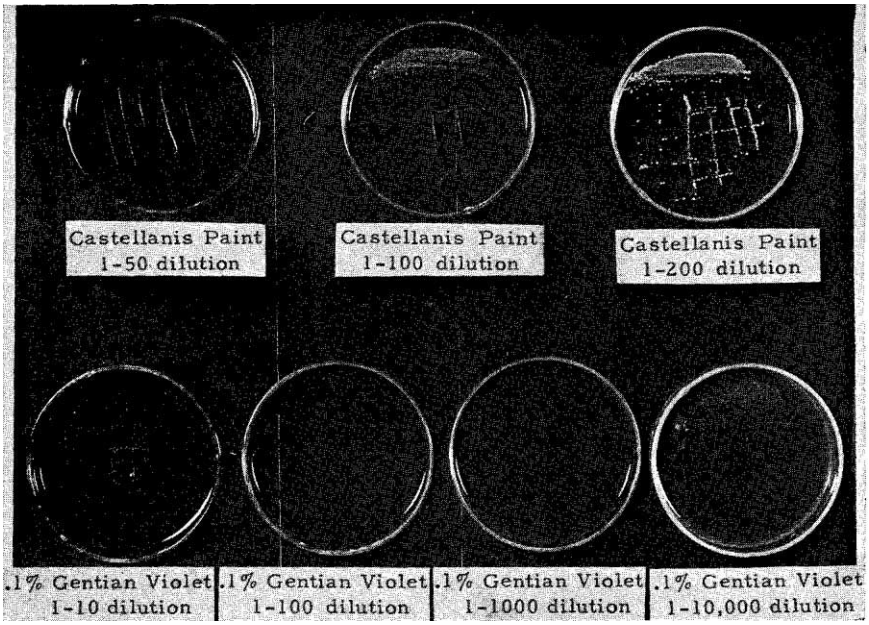

FIG. 1. Sabourauds agar with Castellanis' paint (top row) and 0.1% gentian violet solution (bottom row) showing inhibition of *Candida albicans*.

other attributes. The Castellani's compound solution of carbolfuchsin is used undiluted. The solution has distinct drying qualities and has a definite keratolytic ability.

In our hands, however, the 1 per cent gentian violet in 10 per cent alcohol has given clinical results compatible with the "in vitro" effects here presented.

CONCLUSION

1. A comparative *in vitro* study showing the inhibitory activity of gentian violet solution, Castellani's compound solution of carbolfuchsin, and its individual ingredients on a known virulent strain of *Candida albicans* is presented.

2. Gentian violet solution will inhibit the growth of *Candida albicans* in a dilution of 1–1,000,000 while inhibition with Castellani's compound solution of carbolfuchsin begins at a dilution of 1–100.

TABLE 3

Sabouraud's agar with ingredients of Castellani's paint

	24 HOURS				48 HOURS			
	1–25	1–50	1–100	1–200	1–25	1–50	1–100	1–200
Castellani's Paint	No growth	No growth	No growth	Slight growth	No growth	No growth	Moderate growth	Abundant growth
5 per cent aqueous solution Phenol	No growth	Slight growth	Moderate growth	Abundant growth	No growth	Abundant growth	Abundant growth	Abundant growth
7 per cent alcoholic solution Basic Fuchsin	No growth	No growth	Moderate growth	Abundant growth	No growth	No growth	Abundant growth	Abundant growth
8 per cent Resorcin solution	Slight growth	Moderate growth	Abundant growth	Abundant growth	Slight growth	Abundant growth	Abundant growth	Abundant growth
4 per cent Acetone solution	Abundant growth	Abundant growth	Abundant growth	Abundant growth	Abundant growth	Abundant growth	Abundant growth	Abundant growth
0.8 per cent Boric Acid solution	Abundant growth	Abundant growth	Abundant growth	Abundant growth	Abundant growth	Abundant growth	Abundant growth	Abundant growth

3. The alcoholic solution of basic fuchsin is the most active ingredient in the Castellani's compound solution of carbolfuchsin showing inhibition in the 1–100 dilution. A 5 per cent aqueous solution of phenol showed the next greatest inhibition starting at a 1–50 dilution.

4. Other factors such as drying and keratolytic effect must play a large part in the action of Castellani's compound solution of carbolfuchsin as a therapeutic agent in intertriginous moniliasis since it has such weak inhibitory activity.

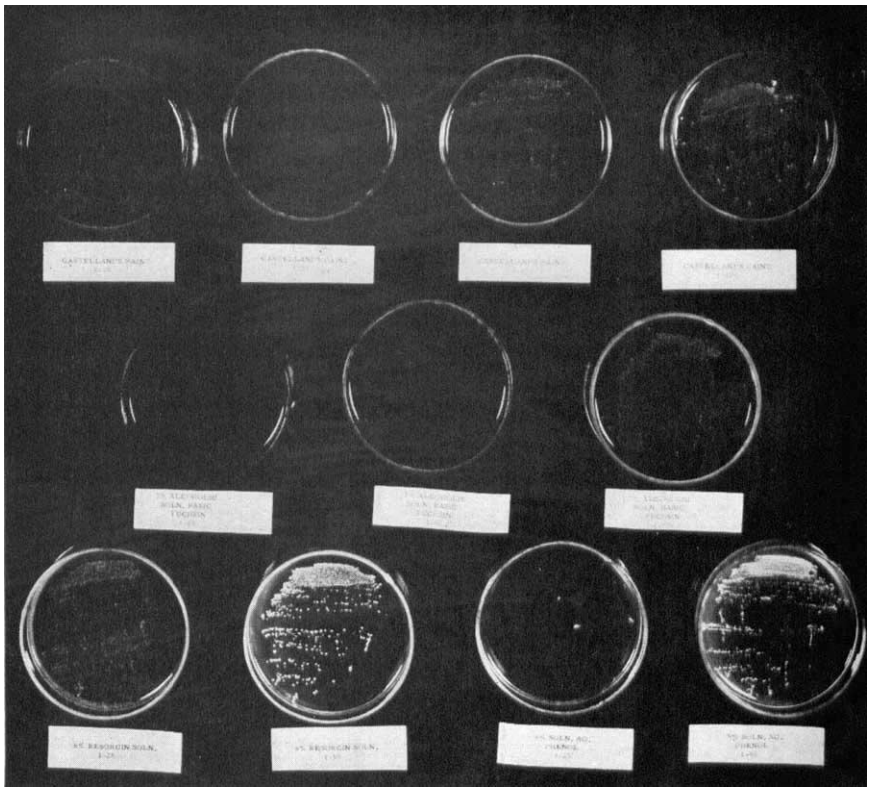

FIG. 2. Sabourauds agar with ingredients of Castellanis paint showing inhibition of growth of *Candida albicans*.

5. In our experience, gentian violet solution is an effective agent for the treatment of intertriginous moniliasis.

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